Acta Biochimica et Biophysica Sinica ABBS

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Journal:	*Acta Biochimica et Biophysica Sinica*
Manuscript ID:	ABBS-2007-060.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	05-May-2007
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Keywords:	Calprotectin , Calcium, Zinc, Circular Dichroism, Thermal stability

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For Review

Human Calprotectin: Effect of Calcium and Zinc on the Secondary and Tertiary Structures, Role of pH in Thermal Stability

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Running title: Human Calprotectin: Effect of Calcium and Zinc on the Secondary and Tertiary Structures, Role of pH in Thermal Stability

Abstract

Calprotectin is a heterodimeric complex, belongs to S100 proteins family and it has been found predominantly in the cytosolic fraction of neutrophils. In the recent study human calprotectin was purified from neutrophil, using a two steps ion exchange chromatography. The purified sample was used for CD (circular dichroism) studies and fluorescence analysis in the presence of physiological concentrations of calcium and zinc, as well as for inhibitory assessment of the protein on K562 leukemia cell line. Also a comparison between thermal stability of the protein in pH 7.0 (physiological pH) and 8.0 (next to intestinal pH) was performed. The results of cell proliferation analysis revealed that human calprotectin initiates growth inhibition of the tumor cells in a dose dependent manner. The intrinsic fluorescence emission spectra of the human calprotectin (50 µg/ml) in the presence of both calcium and zinc ions, indicate a reduction in fluorescence intensity, reflecting a conformational change within the protein with exposure of aromatic residues to protein surface that is important for biological function of calprotectin. The far UV-CD spectra of the human calprotectin in the presence of physiological concentrations of calcium and zinc ions indicate a reduction in α-helical content of the protein and an increase in ß- and other structures. Our results also demonstrate that increasing of pH from 7.0 (physiological pH) to 8.0 (next to intestinal pH) lead to marked elevation in the thermal stability of human calprotectin, showing a significant role of pH in the stability of calprotectin in the gut.

Key words: Calprotectin; Calcium; Zinc; Circular Dichroism; Thermal stability

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